Biomarkers in premanifest Huntington's disease

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Changes in striatal dopamine D2 receptor binding in preclinical Huntington's disease.

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ABSTRACT

Carriers of the Huntington disease (HD) mutation develop a progressive neurodegenerative disorder after a pre-clinical phase. We examined the value of ^{11}C-raclopride PET (RAC) as a biomarker for pre-clinical HD pathophysiology. Methods: In a prospective cohort study with clinical and neuropsychological assessment we collected complete RAC data in 18 pre-clinical mutation carriers (HD-PMC) and 11 controls. Follow-up was 2 years. We calculated striatal RAC binding potential (BP) to measure dopamine D2 receptor availability. Results: No HD-PMC had overt neuropsychological dysfunction. RAC-BP in putamen was abnormal in up to 44% of HD-PMC. The rate of RAC-BP decline (2.6% per year) was not significantly higher than in controls. Follow-up putaminal BP correlated weakly with predicted distance to onset of clinical HD (p = 0.034), but the rate of decline did not. Three HD-PMC developed motor abnormalities suspect for HD but did not show an increased rate of decline of putaminal BP. Conclusions: Many HD-PMC have striatal abnormalities but we found no clearly increased rate of D2 receptor changes around the onset of clinical HD. A longer follow-up of the present study cohort is needed to establish the value of RAC-BP in assessing the risk of clinical conversion from striatal D2 binding data.

INTRODUCTION

Huntington's disease (HD) is an autosomal dominant disorder caused by an expansion of the CAG repeat in the IT15 gene[1]. After a symptom-free period, it leads to progressive impairments in motor, cognitive and psychiatric domains with a mean age of onset around forty. The number of CAG repeats is positively associated with earlier disease onset[2], severity of neuropathologic changes[3,4] and in vivo striatal dopamine D_2 receptor loss[5,6]. D_2 receptors are particularly well expressed on the striatal medium spiny neurons, which have a propensity to be affected early in HD and can be measured by ^{11}C-raclopride (RAC) PET.

Although mutation carriers are exposed to the mutant protein from conception, some pre-clinical mutation carriers (HD-PMC) show normal striatal metabolism and receptor state in PET studies[7], with no evidence of brain atrophy on pathological examination[8]. Uncertainty exists regarding the rate of development of neuronal damage in the pre-clinical disease state, which is traditionally defined as the absence of motor manifestations of HD in mutation carriers.

With a view to testing putative neuroprotective strategies, knowledge about the time course of neuronal impairment and its relation to clinical disease onset is important. A surrogate biological marker is needed to provide an objective way to assess this relationship[9]. Besides (RAC) PET, other methods applied are striatal MRI volumetry[10] and ^{18}F-fluorodesoxyglucose (FDG) PET[11]. MR-spectroscopy seems less promising[12]. A direct comparison of these methods to assess their respective advantages is lacking. The purpose of the present longitudinal study is to elucidate changes in striatal D_2 receptor binding in HD-PMC and to assess whether these changes herald conversion to clinical HD.

METHODS

Subjects

Our study population has been described in detail elsewhere[13]. Briefly, we studied 14 controls and 27 HD-PMC of the Huntington's disease (HD) gene classified to be free of HD signs as indicated by a score of 0 or 1 (meaning no or non-specific motor abnormalities or soft signs) on the Unified Huntington's Disease Rating Scale (UHDRS) scale of clinical confidence[14]. The modeled probability of onset of clinical disease within 5 years[15] (Prob5) was calculated (Table 1). Results of the FDG scans and of the neuropsychological assessment (NPA) will be reported separately. Investigators were blinded to gene status.

Eighteen HD-PMC and 11 controls had complete baseline and follow-up FDG and (RAC) PET

Table 1. Characteristics of study subjects at baseline

	Preclinical Mutation Carriers (n=18)	Controls (n=11)
Age	39.9 ± 7.2 (29-56)	48.4 ± 10.2 (30-65)
No. of females (%)	10 (56)	5 (46)
UHDRS motor score	0.22 ± 0.73 (0-3)	<1
CAGrepeats	43 ± 2.2 (39-47)	<35
Prob5	0.21 ± 0.17 (0.01-0.60)	not calculated
Cognition score abnormal No. (%)	0 (0)	0 (0)
Cognition score low-normal No. (%)	6 (33)	4 (36)

All data are presented as mean ± SD (range) unless indicated otherwise
UHDRS: Unified Huntington's Disease Rating Scale
Prob5: probability of conversion to clinical HD within 5 years

scans that were available for analysis and these subjects are studied in this article. Missing data resulted from withdrawal from the study, technical failures and pregnancy. UHDRS motor scores or Prob5 of the nine HD-PMC with incomplete scan data were not significantly different from the HD-PMC reported here.

HD-PMC were on average 8.5 years younger than controls (Table 1). Follow-up was longer than 2 years in all subjects, with a mean of 2.4 years. The institutional ethics committee approved the study and all subjects gave written informed consent.

DATA ANALYSIS

Positron emission tomography

Dynamic FDG and RAC baseline and follow-up scans were performed on a single Siemens ECAT Exact HR+ scanner (Siemens Erlangen, Germany), following a previously described protocol[13].

The first six frames (for RAC) or the last four frames (for FDG) of each dynamic scan were summed. We used the SPM2 software package (Wellcome Department of Cognitive Neuroscience, Institute of Neurology, London, UK) to co-register each subject's summed

RAC scans to the summed baseline FDG scan and to normalize the ensuing data in one session into the Montreal Neurological Institute standard coordinate system (MNI space) using the summed baseline FDG scan as source image. In this way, we minimized the impact of any biases due to data processing on the in-tra-individual differences between baseline and follow-up scans. Using a dedicated in-house automated soft-ware program, standard automated anatomical labeling (AAL) regions of interest (ROI)[16] were positioned on the co-registered, spatially normalized RAC scans and time-activity curves were extracted. Binding potentials (BP) were calculated in putamen and caudate nucleus applying the simplified reference tissue model[17], using a large AAL cerebellar ROI (labeled Crus_2) on both sides as a reference region.

MRI

On MRI scanning, we found no indication of other CNS disease.

Neuropsychological testing

Baseline NPA was available in 16 of 18 HD-PMC and in all controls. Test results were clustered in three domains: memory, psychomotor speed and executive function/attention. Scores on each domain were considered abnormal if below 2SD of the control mean (M), low-normal if between M-1SD and M-2SD, and normal when above M-1SD of controls. An overall cognition score was calculated and defined as abnormal if the subject scored abnormal on more than one domain, or abnormal on one and low-normal on any other domain. This cognition score was considered low-normal when a subject scored low-normal on any domain, or had an abnormal score on one domain, with normal scores on all other domains.

Statistical analysis of data was performed with the JMP software package (version 5; SAS Institute Inc., Cary, NC, USA). Abnormal results were defined as over 2SD from the control mean. Group differences were tested with analysis of variance (ANOVA), differences over time with a t-test for matched pairs.

RESULTS

At baseline, no subjects had abnormal cognition scores. Six of 18 HD-PMC (33%) and four of 11 controls (36%) had low-normal cognition scores at baseline. Two HD-PMC were not tested at baseline but subsequently had normal cognition scores.

Putaminal RAC activity was significantly lower in HD-PMC compared with controls at baseline (p 0.001) and follow-up ($p < 0.0001$), with 39% of HD-PMC showing abnormal values at baseline and 44% at follow-up (Table 2). Caudate RAC activity was less often decreased. All

Table 2 Putamen and caudate ^{11}C-raclopride binding potential values in pre-clinical mutation carriers and controls

	Pre-clinical mutation carriers (n = 18)		Controls (n = 11)	
	Baseline	Follow-up	Baseline	Follow-up
Putamen Mean ± SD	2.30 ± 0.52	2.14 ± 0.39	2.96 ± 0.38	2.83 ± 0.36
P	0.001[a]	<0.000 [a]		
Caudate Mean ± SD	1.25 ± 0.38	1.16 ± 0.33	1.49 ± 0.26	1.37 ± 0.33
P	P = 0.08[a]	P = 0.1 [a]		
Abnormal [b] putamen N (%)	7 (39)	8 (44)	0	0
Abnormal caudate N (%)	2 (22)	1 (11)	1 (11)	1 (11)
Change, putamen % per year		- 2.6		- 1.8
P		P = 0.74 [c]		

[a]HD-PMC versus controls.
[b]Abnormal: below control mean – 2SD.
[c]Paired t-test for differences in changes between HD-PMC and controls across groups.

controls had normal BP in putamen, one control had low caudate BP.

During the study, putaminal RAC activity decreased significantly both in HD-PMC (p = 0.006, t-tests for matched pairs) at 2.6% per year and in controls (p 0.02) at 1.8% per year (Fig. 1), but the magnitude of change within each individual was not significant between the two groups (p = 0.74).

We found a weak inverse correlation of putaminal BP and predicted probability of onset of clinical HD within 5 yearsbaseline: R^2 = 0.22, p = 0.047 for linear fit (not shown); follow-up: R^2 = 0.25, p = 0.034 (Fig. 2). The rate of putaminal decrease of RAC activity did not correlate with Prob5.

Three HD-PMC developed ‘motor abnormalities that may be HD signs’ (grade 2, 50–89% on the UHDRS scale of clinical confidence) during the 2-year follow-up period. They did not show choreatic signs at baseline, their scores were maximal on the Functional Assessment and Functional Capacity Scales and all were engaged in gainful employment.

Subject 1 was a home nurse with a Prob5 of 0.51, using antihypertensive medication. At baseline, she had increased latency on horizontal saccade initiation and some overshoot. Her neuropsychological cognition score was low-normal. At follow-up, she had eight UHDRS motor score points on oculomotor signs in addition to slight dysdiadochokinesia, slight left sided rigidity and decreased finger tapping and slight/intermittent chorea in the face and hands. Her cognition score remained low-normal. Subject 2 was a clerk with a Prob5 of 0.08, using citalopram for ‘moodiness’. He had no motor abnormalities at baseline. His cognition

score at baseline was normal. At follow-up, he scored two UHDRS motor score points on oculomotor signs, slightly reduced finger tapping on one side with slight rigidity in the other arm, mild bradykinesia and slight/intermittent chorea in the face, left arm and right leg. NPA was normal.

Subject 3 was a financial office worker with a Prob5 of 0.14 who retired during the study. At baseline, he had mild slowing and increased latency of horizontal saccades. He was not tested neuropsychologically at baseline but had a normal cognition score at follow-up. He

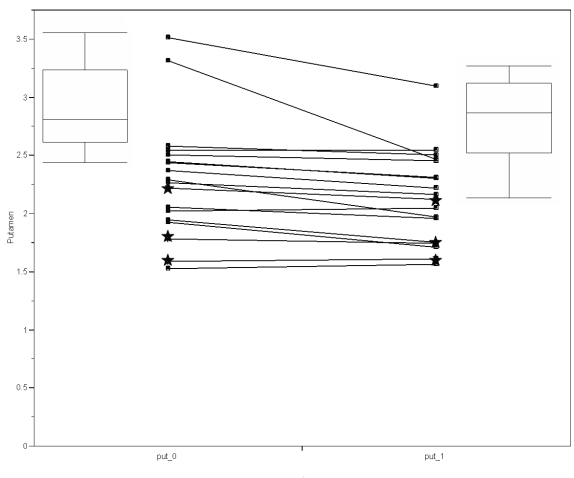

Figure 1 Putaminal RAC activity in pre-clinical mutation carriers. (HD-PMC) at baseline and after at least 2 years of follow-up with control values at baseline (left box plot with median and interquartiles) and at follow-up (right boxplot). Asterisks represent phenoconverting HD-PMC.a

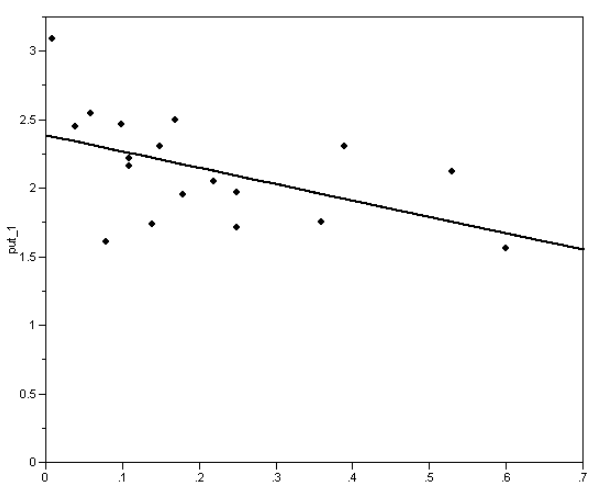

Figure 2 D2 receptor availability in putamen (follow-up scan) decreases with increasing modeled probability of phenoconversion to clinical HD (R2 = 0.25, *p*= 0.034)

then had six UHDRS motor score points on oculomotor signs, mild slowing of finger tapping and slight/ intermittent chorea of the arms and right leg.

Putaminal BP was in the lowest range for two of them and around the HD-PMC mean for the third (Fig. 1). The annual rate of decline was 0.8% in these three subjects and 2.9% for the other HD-PMC (*p* 0.17 on rank sum test).

DISCUSSION

In this longitudinal cohort study, we found that HD-PMC have lower putaminal D_2 receptor availability than controls, both at baseline and after 2 years of follow-up. This lower RAC activity correlated weakly with increasing probability of onset of unequivocal signs of clinical HD (phenoconversion) within 5 years as calculated by an age and CAG repeat based model[15]. Putaminal RAC activity was more sensitive to changes in HD-PMC than caudate.

It is unclear if pathological changes in HD-PMC occur at a constant rate, but this is important in view of the use of PET as a biomarker for future clinical trials[18]. Published data on eight healthy subjects show a calculated decline of relative raclopride distribution volume in basal ganglia of 0.36% per year with wide ranges[19] but another study found an increase of 1.5% per year in putamen RAC-BP in three controls20. In the current study, BP in putamen decreased at 1.8% per year in our 11 control subjects. With PET measurements taking place over a follow-up period of over 2 years, systemic bias can not be excluded. However, the inclusion of a control group scanned at random with the HD-PMC in this same study ascertains the possibility to level out any systemic bias by comparing changes in HD-PMC to controls.

Clinically manifested HD patients have been shown to have constant loss of D_2 receptor availability at around 5% per year[21]. Previous smaller longitudinal RAC studies in HD-PMC have reported annual striatal rates of decline of 6.3%[7] and 4%[20]. In a recent study, with an admirably long follow-up of almost 4 years by Feigin et al., striatal RAC-BP decreased with about 10%[22]. Because no follow-up scans of controls were made, the rate of decline in controls could not be calculated. Thus, the rate of decline in HD-PMC in their study could not be corrected for decreases because of aging or systemic bias, which is diffcult to avoid in long-term PET studies. Of note, the test–retest variability of striatal BP on the same day is estimated at 5%[23]. In our study, HD-PMC showed a decrease in putaminal RAC activity of 2.6% per year (*p* 0.006, t-test for matched pairs). This is consistent with the 10% change found in 4 years in the study by Feigin et al.[22] but was not significantly faster than the change in controls (1.8% per year). We believe that either our approach of PET data processing, apt to detect small intraindividual differences, or the larger sample size provides the explanation for the lower rate of striatal decline we found, compared with the results

of previous studies using operator driven ROI analysis[7,20,22]. Alternatively, if the rate of D_2 receptor loss should change when HD-PMC approach the onset of clinical (motor) HD, HD-PMC group characteristics could also influence this rate of decline. Yet the rate of D_2 receptor loss did not correlate with the predicted proximity to onset of clinical HD. This suggests that there is no acceleration of basal ganglia tissue damage in late-pre-clinical HD. Our study subjects were not close to onset of clinical HD as indicated by the absence of abnormal neuropsychological scores at baseline, their low Prob5 scores and the low number of phenoconverters. Still, many subjects in our study already showed PET abnormalities. Theoretically, HD-PMC may already have a different brain structure from childhood, as the HD mutation is present from conception on. Alternatively, HD-PMC could start with a normal brain structure and continue to suffer losses at a constant but greater rate than with normal aging[4]. Feigin et al.[22] postulated a constant rate of decline and extrapolated their data to calculate that loss of D2 recptors could occur from 25 years before the onset of clinical HD. If however in HD-PMC the pathophysiological D_2 receptor loss would follow an exponential pattern, the rate of decline would be less in subjects closer to phenoconversion. The impression that the three phenoconverting subjects in this study had a rate of decline similar to the other HD-PMC could be in line with this theory.

As we did not correct for atrophy, it is unclear whether the decreased D_2 receptor availability in HD-PMC is because of selective loss of D_2-expressing cells or to a more diffuse process of striatal atrophy. A longer follow-up of the current study population is needed to reliably assess the rate of D_2 receptor loss around phenoconversion. Paulsen et al.[14] studied 'at risk' subjects with a similar mean age as in this study and found conversion from pre-clinical (grade 0 or 1) to unequivocal (grade 3) in 27% in a 2-year period. This appears to be a higher phenoconversion rate than in our smaller cohort. However, CAG repeats in their study subjects were unknown and higher repeat lengths might explain a higher conversion rate.

Another issue regarding the use of RAC as a biomarker is the possibility of a D_2 receptor availability 'threshold' level, below which short-term phenoconversion is likely. Two of three possibly phenoconverted subjects had D_2 receptor availability in the lowest quartile, but the third subject had values around the mean for HD-PMC. Even with these small numbers, our study indicates that there is no sharp dividing line. HD-PMC may differ in their ability to exert compensatory mechanisms during 'pre-clinical' neural damage, which could explain differences in D2 receptor availability at phenoconversion. Possibly, metabolic studies can detect these mechanisms; a recent study indeed reported thalamic activation of an HD-related metabolic pattern[22].

Strengths of this study are the follow-up of over 2 years in a relatively large cohort of HD-PMC with clinical, RAC and neuropsychological data, while a control group was examined in exactly the same way.

Subjects sensing HD symptoms may have an inclination not to take part in a study or to

drop out more often. However, subjects lost to follow-up in this study were not closer to predicted onset of clinical HD and had UHDRS motor scores similar to the HD-PMC reported here. This argues against selective loss of HD-PMC closer to phenoconversion. HD-PMC were younger than controls but this could at most reduce the differences we observed. Controls were sibs of the HD-PMC, without the HD mutation but sharing other genetic and environmental influences. These could theoretically decrease the magnitude of changes found compared with an 'independent' age-matched healthy control group. In addition, our cohort is probably selection biased because all study subjects had chosen to be tested, were asymptomatic and willing to participate in research. This limits the generalizability of our results.

In summary, we found decreased dopamine D_2 receptors in almost half of our well-defined cohort of HD-PMC but no clearly accelerated rate of decline. (RAC) PET does not seem to predict conversion to clinical HD in 2 years in a random group of HD-PMC.

Prospective studies with longer follow-up or with selected HD-PMC with a higher phenoconversion risk are necessary to clarify the neurobiology of HD around the onset of clinical disease and assess the value of RAC as a biomarker for HD in this group.

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REFERENCES

1. The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 1993; 72: 971–983.
2. Kieburtz K, MacDonald M, Shih C, et al. Trinucleotide repeat length and progression of illness in Huntington's disease. Journal of Medical Genetics 1994; 31: 872–874.
3. Furtado S, Suchowersky O, Rewcastle B, Graham L, Klimek ML, Garber A. Relationship between trinucleotide repeats and neuropathological changes in Huntington's disease. Annals of Neurology 1996; 39: 132–136.
4. Penney JB Jr, Vonsattel JP, MacDonald ME, Gusella JF, Myers RH. CAG repeat number governs the development rate of pathology in Huntington's disease. Annals of Neurology 1997; 41: 689–692.
5. Antonini A, Leenders KL, Eidelberg D.11Craclopride-PET studies of the Huntington's disease rate of progression: relevance of the trinucleotide repeat length. Annals of Neurology 1998; 43: 253–255.
6. Sanchez-Pernaute R, Kunig G, del Barrio AA, de Yebenes JG, Vontobel P, Leenders KL. Bradykinesia in early Huntington's disease. Neurology 2000; 54: 119–125.
7. Antonini A, Leenders KL, Spiegel R, et al. Striatal glucose metabolism and dopamine D2 receptor binding in asymptomatic gene carriers and patients with Huntington's disease. Brain 1996; 119: 2085–2095.
8. Gomez-Tortosa E, MacDonald ME, Friend JC, et al. Quantitative neuropathological changes in presymptomatic Huntington's disease. Annals of Neurology 2001; 49:29–34.
9. Shoulson I. Experimental therapeutics of neurodegenerative disorders: unmet needs. Science 1998; 282: 1072–1074.
10. Aylward EH, Sparks BF, Field KM, et al. Onset and rate of striatal atrophy in preclinical Huntington disease. Neurology 2004; 63: 66–72.
11. Feigin A, Leenders KL, Moeller JR, et al. Metabolic network abnormalities in early Huntington's disease: an(18)FFDG PET study. Journal of Nuclear Medicine 2001; 42: 1591–1595.
12. van Oostrom JC, Sijens PE, Roos RA, Leenders KL. 1H magnetic resonance spectroscopy in preclinical Huntington disease Brain Research 2007; 1168: 67–71.
13. van Oostrom JC, Maguire RP, Verschuuren-Bemelmans CC, et al. Striatal dopamine D2 receptors, metabolism, and volume in preclinical Huntington disease. Neurology 2005; 65: 941–943.
14. Paulsen JS, Zhao H, Stout JC, et al. Clinical markers of early disease in persons near onset of Huntington's disease. Neurology 2001; 57: 658–662.
15. Langbehn DR, Brinkman RR, Falush D, Paulsen JS, Hayden MR. A new model for prediction of the age of onset and penetrance for Huntington's disease based on CAG length. Clinical Genetics 2004; 65: 267–277.
16. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. Neuroimage 2002; 15: 273–289.
17. Lammertsma AA, Hume SP. Simplified reference tissue model for PET receptor studies. Neuroimage 1996; 4: 153–158.
18. Paulsen JS, Hayden M, Stout JC, et al. Preparing for preventive clinical trials: the Predict-HD study. Archives of Neurology 2006; 63: 883–890.
19. Schlosser R, Brodie JD, Dewey SL, et al. Long-term stability of neurotransmitter activity investigated with 11C-raclopride PET. Synapse 1998; 28: 66–70.
20. Andrews TC, Weeks RA, Turjanski N, et al. Huntington's disease progression. PET and clinical observations. Brain 1999; 122: 2353–2363.
21. Pavese N, Andrews TC, Brooks DJ, et al. Progressive striatal and cortical dopamine receptor dysfunction in Huntington's disease: a PET study. Brain 2003; 126: 1127–1135.
22. Feigin A, Tang C, Ma Y, et al. Thalamic metabolism and symptom onset in preclinical Huntington's disease. Brain 2007; 130: 2858–2867.
23. Mawlawi O, Martinez D, Slifstein M, et al. Imaging human mesolimbic dopamine transmission with positron emission tomography: I. Accuracy and precision of D(2) receptor parameter measurements in ventral striatum. Journal of Cerebral Blood Flow and Metabolism 2001; 21: 1034–1057.